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A Novel Method for Harvesting Concentrated Platelet Rich Fibrin (C-PRF) with a 10-fold increase in Platelet and Leukocyte Yield

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Running title: Concentrated-PRF (C-PRF): a new concept in liquid PRF therapy

One sentence summary: Concentrated Platelet rich fibrin (C-PRF) is a new liquid-PRF therapy ~10 times more concentrated in platelets/leukocytes when compared to traditional injectable-PRF (i-PRF).

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Abstract

Background: Recently our group proposed a novel method to investigate cell separation following centrifugation of platelet rich fibrin(PRF) using 1mL sequential layers. Owing to these novel findings, it was revealed that cell accumulation following a leukocyte and platelet rich fibrin(L-PRF) protocol (2700RPM for 12 minutes; ~700g) demonstrated a massive accumulation of cells directly above the red corpuscle layer (buffy coat) whereas injectable-PRF (i-PRF) protocols (800 RPM for 3 minutes; ~60g) revealed only a slight shift in platelet/leukocyte concentrations following centrifugation. The purpose of this study was to develop a novel harvesting technique for liquid-PRF with highly-concentrated formulations of platelets/leukocytes.

Methods: Standard high g-force L-PRF and low g-force i-PRF protocols were utilized to separate blood layers. Above each of the red blood corpuscle layers, sequential layers of 100uL were harvested (12 layers total; ie 1.2 mL which represents the total i-PRF volume) and 3 layers (3x100uL) harvested from the red blood cell layer were collected. Each layer was then sent for complete blood count (CBC analysis) and investigated for cell numbers.

Results: The i-PRF protocol revealed typically a 2-3 fold increase in platelet and l.5-fold increase in leukocyte concentration throughout the 1-1.2mL plasma layer when compared to whole blood. While relatively no cells were found in the first 4mL layer of L-PRF, a massive accumulation of platelets and leukocytes were found within the buffy coat with extremely concentrated cells 0.3-0.5mL (~20-fold increases) directly above the red corpuscle layer. We therefore proposed harvesting this 0.3mL and 0.5mL layer directly above the red blood cell corpuscle as a liquid concentrated-PRF (C-PRF). While in general, i-PRF was able to increase platelet numbers by ~250%, we demonstrated that a general 1200-1700% increase in platelet numbers could easily be achieved by harvesting 0.3-0.5mL of C-PRF (total platelet concentrations of >2000-3000x10^9 cells/L).

Conclusions: While conventional i-PRF protocols demonstrate an ability to increase platelet yield by 2-3 fold and leukocytes by 50%, we demonstrate convincingly an ability to concentrate platelet and leukocytes over 10-fold by harvesting specifically by collecting 0.3-0.5mL of C-PRF within the buffy coat.

Keywords: Fibrin, Blood platelets, Regeneration, Wound healing, fibroblasts, platelet rich fibrin

Introduction

Platelet concentrations have been utilized in dentistry and medicine for over 3 decades owing to their ability to concentrate platelets and release supra-physiological doses of growth factors.[1, 2] Platelet rich plasma (PRP) was first developed having widespread use not only in regenerative dentistry, but also in maxillofacial surgery, orthopaedic surgery and esthetic medicine.[3-7] Proper protocols utilizing PRP are typically achieved using anti-coagulants and high g-forces to selectively layer blood cells based on density. Owing to these high g-forces, a top platelet-poor layer (acellular) is typically produced within the upper layer followed by a platelet-rich layer (buffy coat; middle layer), and a bottom red blood cell (corpuscle) layer (Figure 1A). Owing to the use of anti-coagulants, cell layers are separated without fear of coagulation and typical centrifugation cycles typically range from 15 minutes to 1 hour. Despite the success of PRP, various concerns have been raised owing to the use of anti-coagulants which have been shown to negatively impact tissue regeneration.[3, 8, 9]

Platelet rich fibrin (PRF) was therefore developed as a first platelet concentrate with the aim of anti-coagulant removal.[10] As a result, spin cycles are typically much shorter. Following centrifugation, a typical three-dimensional fibrin matrix is produced that may serve as a tissue engineering scaffold utilizing in medicine for various procedures necessitating either soft or hard tissue regeneration.[11-13] In the dentistry alone, PRF has been utilized for the treatment of extraction sockets,[14-17] gingival recessions,[18-20] palatal wound closure,[21-23] regeneration of periodontal defects[24] and hyperplastic gingival tissues.[25] In other medical fields, PRF has been utilized for the successful management of hard-to-heal leg ulcers including diabetic foot ulcers, venous leg ulcers and chronic leg

ulcers.[26] Reported advantages include faster healing, increases in angiogenesis, lower costs (when compared to PRP) and complete immune-biocompatibility.[27-30]

By utilizing even shorter centrifugation cycles and by modifying centrifugation tubes, our group demonstrated the advantages of an injectable-PRF (termed-i-PRF) when compared to PRP.[31] Furthermore, an array of studies has demonstrated the cellular activity of i-PRF superior when compared to PRP.[31-34]

Recently our research group has developed a novel technique to quantify cells within platelet concentrates following centrifugation by sequentially pipetting 1mL layers of blood following centrifugation (under review). Each 1mL layer is then sent for complete blood count (CBC) (Figure 2). This highly effective method to reveal the exact cell position of various cell types following centrifugation allows the direct investigation of the effects of various centrifugation protocols on final PRF cell composition. Our research group surprisingly found that following L-PRF protocols, cells were massively accumulated at the buffy coat directly above the red blood cell layer (within 1mL) with very little cells found throughout the membrane. We therefore aimed to address two specific questions in the current study: 1) In what total volume were the majority of these cells located above the red blood corpuscle layer within the buffy coat and 2) What final concentration could be harvested by selecting collecting the cells found within this precise 'buffy coat' region when compared to conventional i-PRF protocols.

Materials and methods

Preparation of PRF

Blood samples were collected with the informed consent from 6 volunteer donors. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No ethical approval was required for this study because human samples were not identified, as previously described.[35] The factors that affect fibrin clot formation and structure include genetic factors, acquired factors (such as abnormal concentration of thrombin and factor XIII in plasma, blood flow, platelet activation, oxidative stress, hyperglycemia, hyper-homocysteinemia, medications, and cigarette smoking), and other parameters (such as microgravity, pH, temperature, reducing agents, and concentration of chloride and calcium ions).[36] All patients with any of the above conditions were excluded. All patients were included if systemically healthy, non-smoking, and not taking any medications.

The following two centrifugation devices were utilized in this study including the IntraSpin Device‖ and The Duo Quattro¶. Two separate protocols were tested. On the Intraspin device, a ~700 RCF-max (~400 RCF-clot) for 12 minutes was utilized for the L-PRF protocol whereas a ~60 RCF-max for 3 minutes was utilized to produce i-PRF.

Each of the 6 volunteers donated 3 blood collection tubes (10mL plastic tubes) for each of the 2 tested groups for a total of 6 tubes per participant. Additionally, 1 blood sample was collected to quantify control whole blood. Specific plastic hydrophobic tubes# were utilized to minimize/prevent clotting during centrifugation.

For one participant, an additional 2 tubes were harvested to demonstrate the general location of cells following centrifugation using L-PRF and i-PRF protocols first utilizing the previously proposed 1mL sequential pipetting method (data currently under review; presented in Figures 3 as an overview). Since we have previously observed a massive cell accumulation within the buffy layer in a 1mL sample range following the L-PRF protocol, we aimed to investigate precisely the volume in which these cells were located within this 1mL layer directly above the red cell corpuscle layer. As such, we developed a novel methodological approach whereby 100uL sequential layers were pipetted starting from ~1.2-1.5mL layers above the buffy coat down to the red blood cell layer (Figure 4, depicted as +1 to +12 layers). Additionally, 3 layers were harvested within the red blood cell layer to determine the number of cells incorporated within this layer as well (Figure 4, depicted as -1 to -3 layers). Each of these 100ul layers was sent for CBC analysis.

The second tube from each group was utilized to determine the final concentration from the liquid version of the i-PRF yellow plasma layer and to compare results to the average sequential 100ul layers. For L-PRF protocols, one tube was utilized to harvest 0.5mL of a concentrated PRF (defined as the 0.5mL buffy coat directly above the red blood corpuscle). This layer was termed concentrated-PRF (C-PRF) throughout the article in reference to the harvesting of this concentrated buffy coat layer. Similarly, a 0.3mL of C-PRF liquid was harvested from this layer as well. Blood draw for 100uL sequential analysis was carried out with anti-coagulants to allow for blood samples to thereafter be sent for complete blood counts (CBC) where the total number of leukocytes, red blood cells, platelets, neutrophils, lymphocytes and monocytes were calculated from each sample. Thereafter, each sample was displayed graphically using GraphPad Prism 6.0 software**.

Results

Comparison of cell separation following centrifugation at L-PRF and i-PRF protocols

First, a novel method to investigate cell types following centrifugation utilizing 1mL sequential pipetting was utilized to investigate in which layers the various cell types were found following centrifugation at both high (L-PRF) and low (i-PRF) g forces (Figure 2, 3). Interestingly, it was first revealed that the L-PRF protocol accumulated the majority of leukocytes and platelets, monocytes and lymphocytes in layer 5; the layer representing the buffy coat transition between the yellow and red blood cell layers (represented by arrows in Figure 3A). Within this layer, a general 3 to 5 fold increase in cell concentration was observed when compared to control whole blood values; represented on the left hand side of each graph (Figure 3A). In contrast, the i-PRF protocol demonstrated a 1.5 to 2.5 general fold increase in various cell types in layers 1-2 (Figure 3B). While the total concentration of i-PRF was certainly richer in total platelets and leukocytes when compared to the L-PRF protocol (owing to the fact the i-PRF volume was ~1-1.3mL vs the 4.5-5mL found in L-PRF), we therefore sought to collect simply the layer within the buffy coat representing a massive increase in cell number.

Sequential pipetting of 100ul layers within i-PRF and L-PRF

The first question that our group planned to address in the present study was up to what volume within the buffy coat following the L-PRF protocol was this massive increase in cell numbers observed. To address this question, removal of the first 3.5mL of the upper plasma layer was removed (acellular layer) from the centrifugation tube (leaving 1.0-

1.5mL of remaining sample above the red blood cell layer). Thereafter a sequential pipetting methodology was once again utilized with 100uL layers to accurately determine up to what layer above the red cell layer above the L-PRF plasma layer were the cells located (Figure 4). Furthermore, 300uL within the red blood corpuscle layer were also harvested and quantified in 100uL sequential layer (Figure 4). In contrast, the entire i-PRF layer was collected starting from the upper 100uL layer and sequentially pipetted until all plasma layers were collected (Figure 4). Once again, 300uL was sequentially pipetted in 100ul layer from the red blood corpuscle layer.

Figure 5A demonstrates the results following sequential 100uL layers of the i-PRF protocol. Notice how specifically in layer 13, a 3 fold increase (from 5 to 15x109cells/L) is found in leukocytes directly at the buffy coat layer 13 (represented by arrows). Notice also the 5-6 fold increase in monocytes. Notice how the red blood cells begin to accumulate at layer 13 and by layer 14 demonstrate that the sample is now within the red blood layer. Notice how the remaining white blood cells and platelet levels drop within layer 14 after the yellow-red transition (Figure 5A). Followin the i-PRF protocol, we observed a 2.5 fold increase (from baseline ~220 to ~550 x10^9 platelets/L) in platelets in all 13 layers from this i-PRF protocol (1.3 mL ie 13 time 100uL samples) and only a slight increase in leukocytes.

Figure 5B demonstrates the results following sequential 100uL layers above the red blood layer found following the L-PRF protocol. Interestingly almost all the cells accumulate within 3 layers (ie 300uL) above the red blood corpuscle (Figure 5B). Most surprisingly, within this layer, a massive increase in platelets, monocytes, leukocytes and lymphocytes. For instance, a roughly 225 to 6000 x x10^9 platelets/L increase is observed

representing a >25 fold increase in platelet concentration; specifically 100ul above the red blood cell layer.

Based on these results, we assumed a 0.3 to 0.5mL layer of concentrated-PRF (C-PRF) could be preferentially collected within this buffy coat directly above the red cell layer (Figure 1B). Figure 6A demonstrates that while the i-PRF protocol increases leukocyte numbers 1.23 fold, a marked and significant increase representing a 4.62 and 7.34 fold increase were observed with both 0.5 mL and 0.3 mL C-PRF respectively. Even more pronounced, while i-PRF protocols have typically been shown to increase platelet yields between 200 and 300%, the C-PRF protocols massively increased platelet yields 1138% and 1687% respectively (Figure 6B). A similar trend was also observed for monocytes (Figure 6C). Total values following averages from 6 patients are summarized in Table 1.

Discussion

Around the year 2014-2016, it was the understanding that the low speed centrifugation concept (also referred to as the LSCC) could achieve maximum amounts of cells and growth factors following centrifugation owing to the reduced relative g-force applied during centrifugation (ie less cell pushed towards the bottom of centrifugation tubes). When investigating full-sized PRF membranes or plasma layers, this proved to be true and confirmed in numerous studies to date.[37-40] Furthermore, our group further showed that centrifugation utilized lower centrifugation speeds (now termed advanced-PRF) was further shown to release higher total growth factor release of PDGF, TGF-β1, VEGF, EGF and IGF when compared to control L-PRF.[41, 42]

The findings from the present study represent a paradigm shift in our understand of liquid PRF and the ability to further concentrate platelets and leukocytes in C-PRF in the proposed method utilized in this study. This represents a major breakthrough in the differences reported between PRP, i-PRF, and C-PRF in platelet yields. Many clinicians believe that platelet yields upwards of 1500 x10^9 cells/L are ideal for regenerative purposes yet i-PRF protocols were typically found to accumulate cells within the 500-600 x 10^9 cells/L. One of the main advantages of i-PRF was the fact that owing to its anti-coagulant removal, clotting occurs shortly following injection or when mixing with biomaterials. We demonstrated recently that this clotting allows for a more slow and gradual release of growth factors over time when compared to PRP.[43] Nevertheless, a comparative study between i-PRF and PRP demonstrated very similar growth factor release over time with some growth factors being more released in i-PRF whereas other in PRP.[35] Interestingly, in that study, it was found that when platelet concentrates were compared during in vitro cell cultures, i-PRF produced significantly greater cell activity when compared to PRP.[35]

Within the past few months however, by utilizing the sequentially pipetting methodology to accurately observe the effect of centrifugation protocols on separation of cell layers, it was very accurately shown the influence of L-PRF protocols on accumulation of platelets and leukocytes at the buffy coat. While A-PRF protocols centrifuged at 200 RCF-max (as opposed to 700 in L-PRF) have been shown to more evenly distribute platelets in the upper 4mL layers of the plasma layer (data currently under review), it was extremely interesting to note the massive accumulation of platelets and leukocytes directly above this red blood cell layer. We therefore aimed to 1) investigate first in what volume the majority of these cells were located, and 2) at what concentration could we maximize

the concentration of leukocytes and platelets in PRF by harvesting this specific concentrated region of cells (C-PRF).

One of the surprising findings was how tightly located the various cells were located directly above the red blood cell layer. In all samples, cells were routinely located within 0.3-0.5mL above the red blood cell corpuscle with the great majority located directly 0.1mL above. Noteworthy, a large ratio of leukocytes and monocytes were located within the first 100uL red blood cell layer and it remains of interest to determine whether this 100uL of red blood cells should be collected owing to their massive inclusion of various white blood cells when compared to baseline. In contrast, though L-PRF has also been given the tradename 'leukocyte' and platelet rich fibrin (ie L-PRF), it is interesting to point out that leukocyte concentration in L-PRF are actually lower than control whole blood. This means that following centrifugation to produce L-PRF membranes, an actual ***decrease*** in leukocyte numbers is observed. The protocol with the greatest ability to concentrate leukocytes (i-PRF) only increases their concentration by roughly 50%. Within the present study, we demonstrate for the first time an ability to concentrate leukocytes between 500-750% when compared to whole blood by utilizing C-PRF protocols. We also demonstrated an ability to concentrate platelets over 15 fold when compared to baseline whereas i-PRF protocols are only able to concentrate platelets 2-3 fold (Table 1, Figure 6).

One interesting phenomenon left to investigate is the further optimization of the C-PRF protocol. In general, L-PRF protocols are known to push the majority of leukocytes and other white blood cells (greater than 50%) within the red blood cell layer (not include in the PRF layer). Therefore, it would be interesting to further evaluate to determine if a better yield of platelets and leukocytes could further be obtained by modifying

centrifugation protocols. Another interesting recent development has been the better ability to separate cell layers (based on density) using horizontal centrifuge. A further study comparing horizontal centrifugation versus fixed-angle centrifugation is also needed to potentially further improve C-PRF protocols. In this study, we have developed a new method to harvest the rich portion (more cells/growth factors) from liquid blood concentrates.

Conclusion

The results from the present study demonstrate a novel technique for investigating cell accumulation directly above the red blood cell layer within the buffy coat. It was revealed that a massive accumulation of leukocytes, platelets and monocytes were located specifically within this 0.3-0.5mL layer when utilizing high g-force protocols (L-PRF). While conventional i-PRF protocols are able to concentrate leukocytes up to 1.5 fold and platelets 2-3 fold, we demonstrates a >500% increase in leukocytes and >1500% increase in platelets by utilizing this novel concentrated-PRF (C-PRF) technique. Further comparative pre-clinical and clinical studies are now needed to determine the added regenerative potential of C-PRF when compared to conventional protocols.

Compliance with ethical standards

Conflict of interest

All authors declare no conflict of interest.

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Ethical Approval
No ethical approval was required for this study as human samples were not identified in this study.

Informed consent
For this type of study, informed consent was not required as no human or animal subjects were utilized.

‖ IntraLock, Boca Raton, Florida, USA
¶ Process for PRF, Nice, France
# Chixin Biotech,Wuhan,China
** GraphPad Software, Inc., La Jolla, CA, USA

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Figure Legends

Figure 1: (A) Illustration demonstrating typical blood cell separation following high-speed centrifugation to produce PRP. Typically an acellular platelet poor plasma (PPP) layer is observed above a platelet-rich 'buffy-coat'. Below this is the red blood corpuscle layer. **(B)** Proposed method to harvest concentrated-PRF (C-PRF). Following L-PRF protocols, since all cells are accumulated within 300-500uL above the red cell layer, it is proposed to collect 0.3-0.5mL of liquid C-PRF directly above the red cell junction for a highly-concentrated liquid of platelets, leukocytes and monocytes.

Figure 2: Illustration demonstrating the proposed novel method to quantify cell types following centrifugation. Currently, one of the limitations is that whole blood is compared to the total plasma concentration following centrifugation. This however, does not give a proper representation regarding the location of cells following centrifugation. By utilizing the proposed technique in this study by sequentially pipetting 1mL of volume from the top layer downwards, it is then possible to send each of the 10 samples for CBC analysis and accurately determine the precise location of each cell type following centrifugation at various protocols. Notice that one layer (in this case layer 5) will contain some yellow plasma and red blood cells. This is typically the location of the buffy coat where a higher concentration of platelets is typically located.

Figure 3: (A) The concentration of cell types in each layer from 1mL down to the 10^{th} mL sample utilizing the L-PRF protocol (2700RPM for 12 minutes; ~700g). Notice that the majority of platelets, leukocytes and monocytes accumulate directly within the 5^{th} layer in

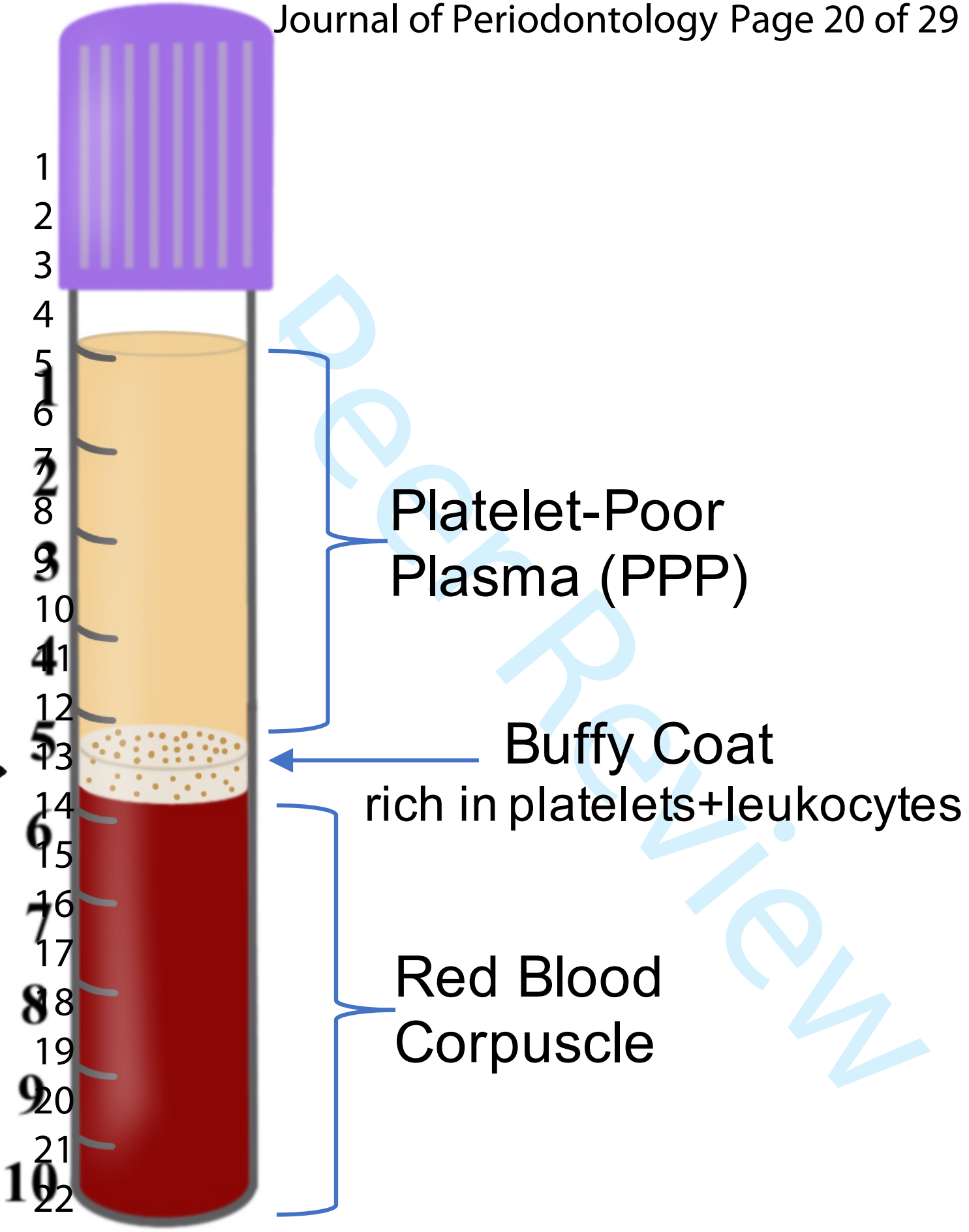
the buffy coat. The first 4 layers of this plasma layer, was typically devoid of all cells. **(B)** The concentration of cell types in each layer from 1mL down to the 10th mL sample utilizing the injectable i-PRF protocol (800RPM for 3 minutes; ~60g). Notice that very little change in platelet or leukocyte accumulation is observed utilizing this centrifugation cycle. A slight increase in platelets and leukocytes is however observed when compared to control in the upper 1-2 mL layers.

Figure 4: A second methodological illustration depicting the sequential harvesting technique. Briefly, as it was revealed that the majority of cells accumulate within the 1mL within the buffy coat, we then sought to investigate precisely the total volume of liquid (mL) above the buffy coat cells are concentrated. For the L-PRF protocols, 3.5mL were removed followed by sequential 100uL layer pipetted followed by CBC analysis. 3 layers in the red blood cell layer were also harvested. In comparison, all plasma layers of the i-PRF protocol were also harvested in 100ul sequential layers. 3 red blood cell layers (100uL each) were also collected.

Figure 5: (A) The concentration of cell types in each layer from 100 uL layers in i-PRF and 3 layers into the red cell layer. Notice that platelets tend to increase 2-3 fold where a slight increase in leukocytes and other cell types is observed directly at the separating layer between plasma and red blood cell layers. **(B)** The concentration of cell types in each of the 12 layers (100 uL each) above the red blood cell layer following L-PRF protocol. Notice the massive increase in platelets (roughly a 20 fold increase specifically at the buffy coat

layer between the yellow and red blood cell layers. Interestingly, all cells seem to accumulate within 3-5 layers (300-500uL) above the red blood corpuscle only.

Figure 6: Concentration of (A) leukocytes (B) Platelets and (C) Monocytes following centrifugation using i-PRF protocols versus collecting 0.3-0.5 mL of concentrated-PRF (C-PRF). Notice that while i-PRF was typically responsible for a 1.2-2.5 fold increase in the various cell types following centrifugation, up to a 15 fold increase in platelet concentration could be achieved with C-PRF.


Platelet-Poor
Plasma (PPP)
Buffy Coat
rich in platelets+leukocytes
Red Blood
Corpuscle

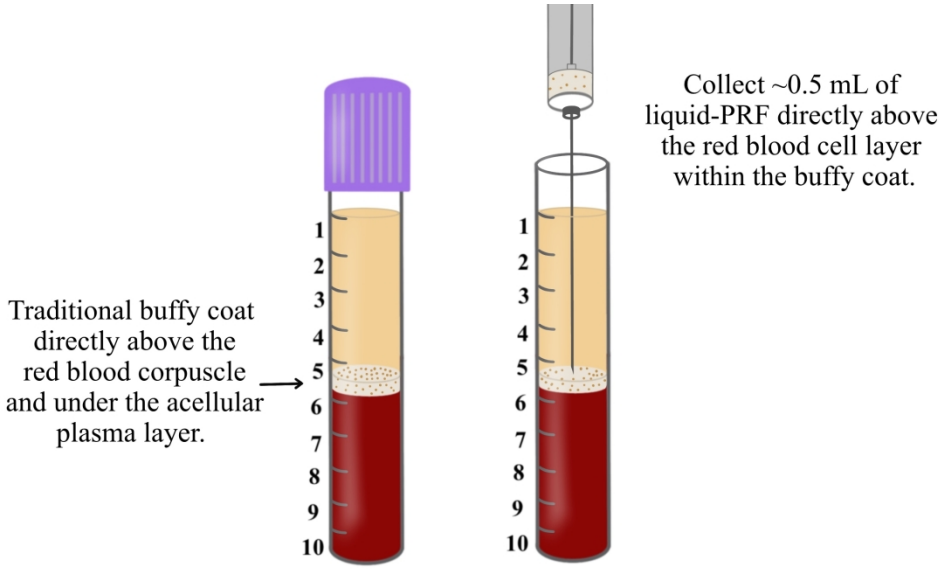


Figure 1(B) Proposed method to harvest concentrated-PRF (C-PRF). Following L-PRF protocols, since all cells are accumulated within 300-500uL above the red cell layer, it is proposed to collect 0.3-0.5mL of liquid C-PRF directly above the red cell junction for a highly-concentrated liquid of platelets, leukocytes and monocytes.

215x139mm (300 x 300 DPI)

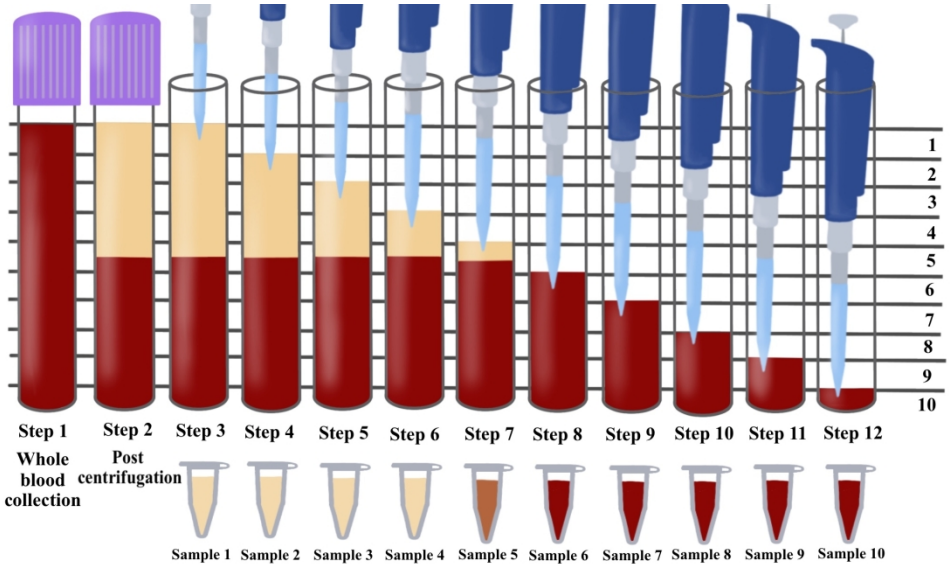


Figure 2: Illustration demonstrating the proposed novel method to quantify cell types following centrifugation. Currently, one of the limitations is that whole blood is compared to the total plasma concentration following centrifugation. This however, does not give a proper representation regarding the location of cells following centrifugation. By utilizing the proposed technique in this study by sequentially pipetting 1mL of volume from the top layer downwards, it is then possible to send each of the 10 samples for CBC analysis and accurately determine the precise location of each cell type following centrifugation at various protocols. Notice that one layer (in this case layer 5) will contain some yellow plasma and red blood cells. This is typically the location of the buffy coat where a higher concentration of platelets is typically located.

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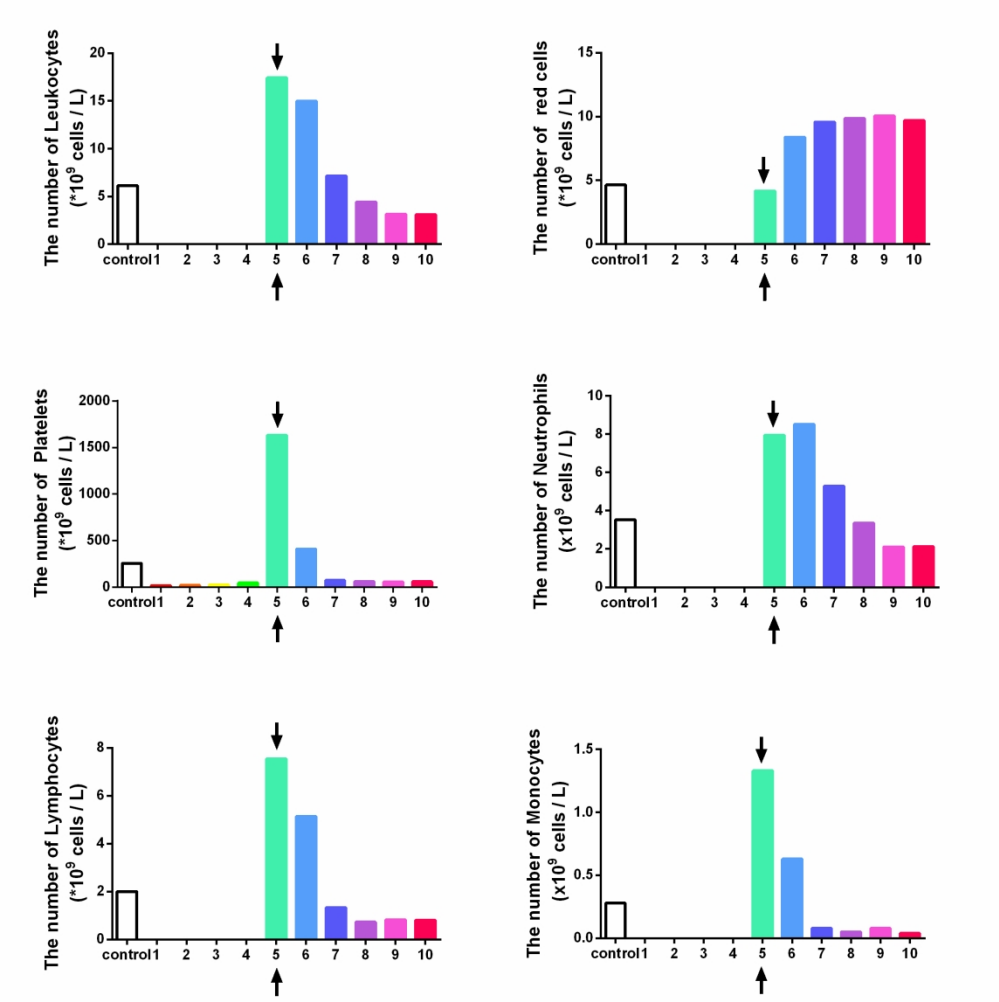

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201x206mm (300 x 300 DPI)

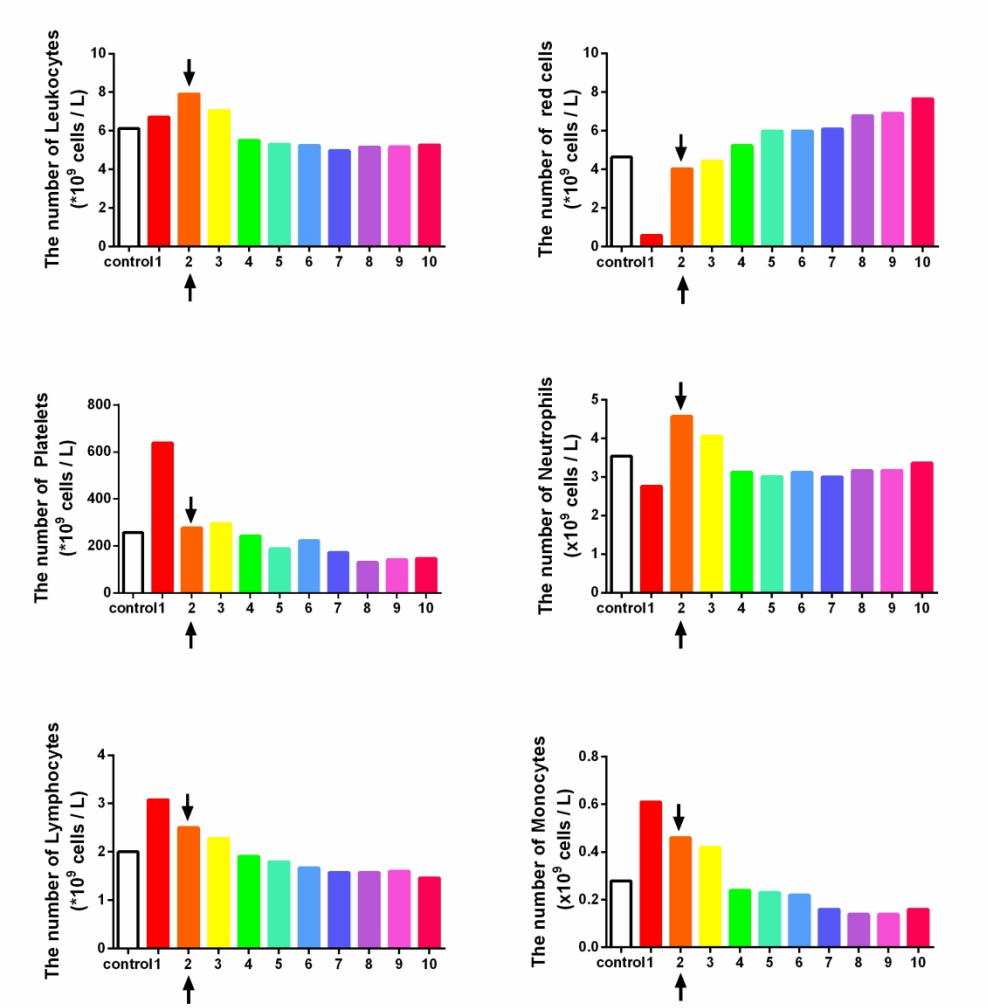

Figure 3(B) The concentration of cell types in each layer from 1mL down to the 10th mL sample utilizing the injectable i-PRF protocol (800RPM for 3 minutes; ~60g). Notice that very little change in platelet or leukocyte accumulation is observed utilizing this centrifugation cycle. A slight increase in platelets and leukocytes is however observed when compared to control in the upper 1-2 mL layers.

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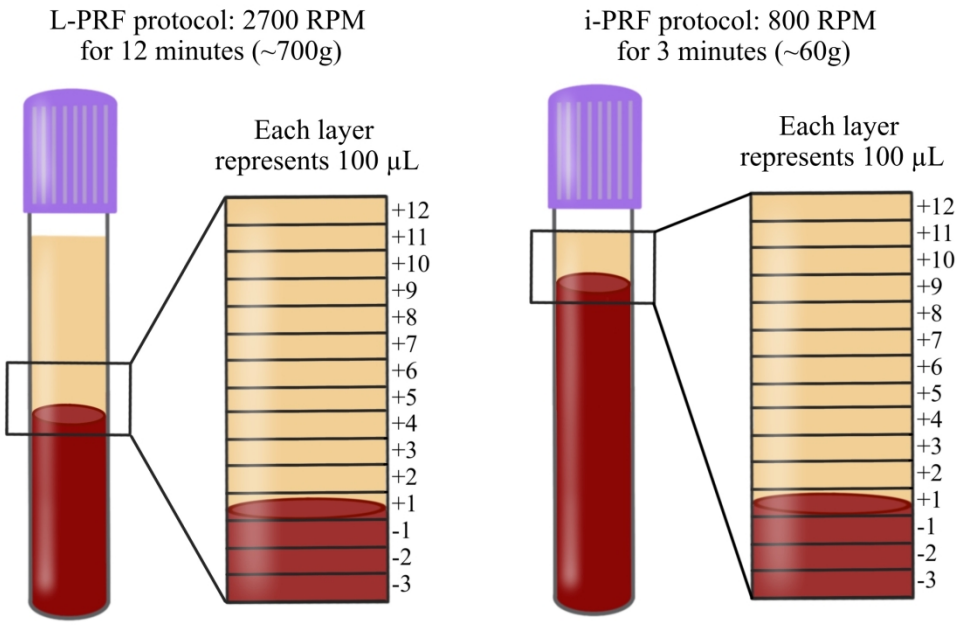

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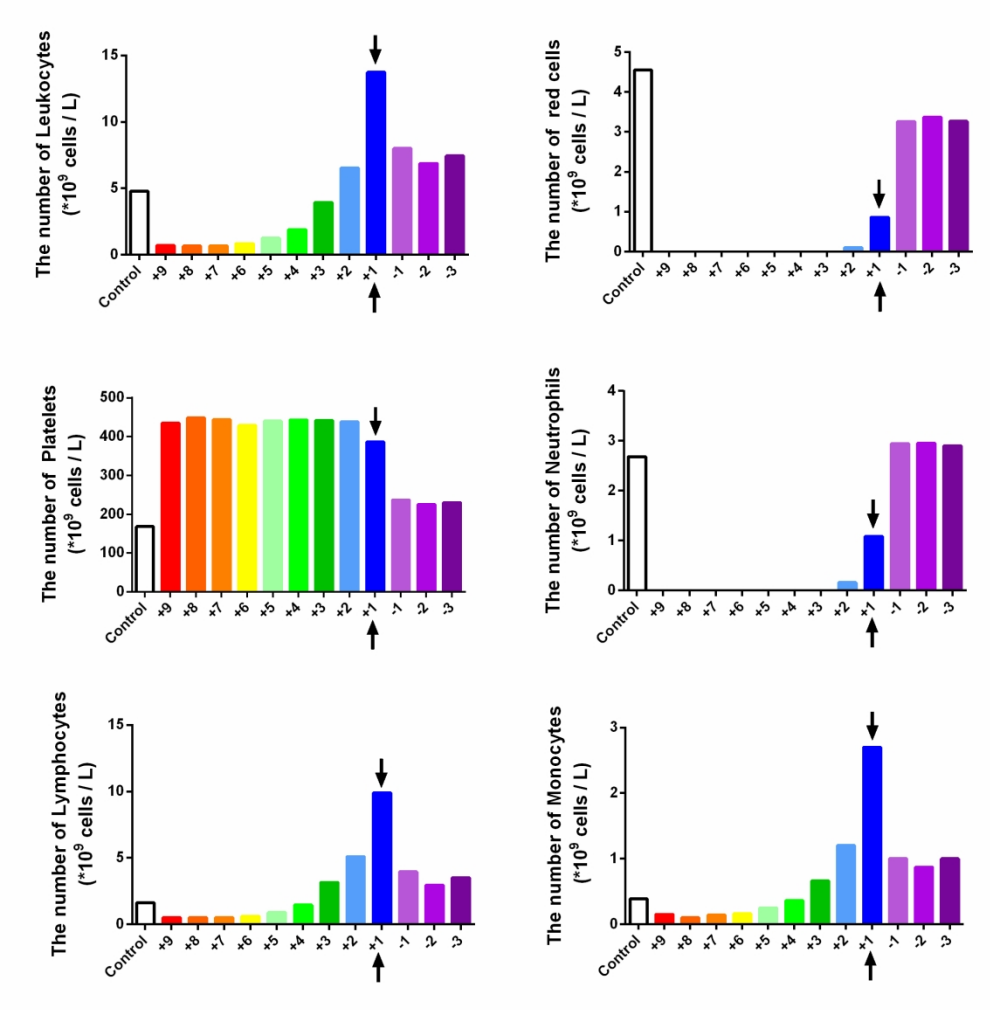
215x139mm (300 x 300 DPI)



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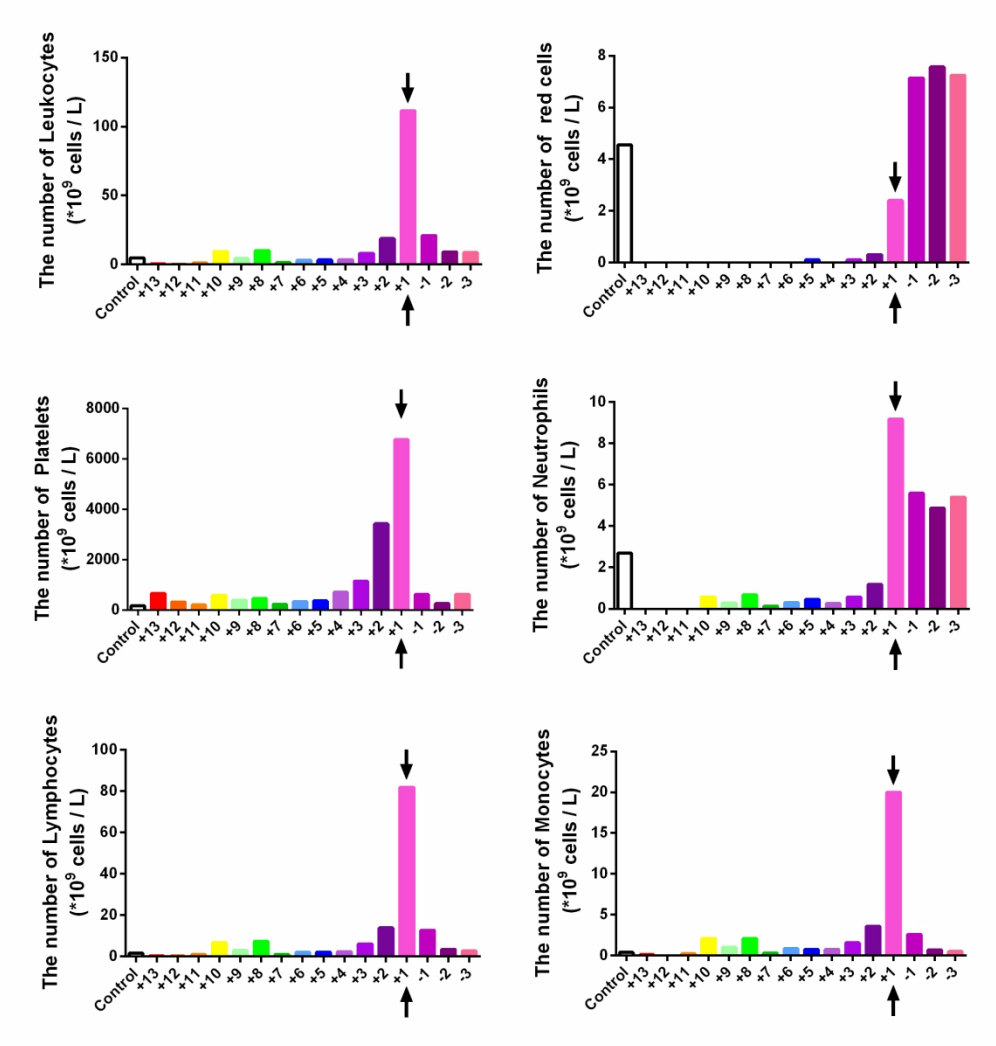

Figure 5 (B) The concentration of cell types in each of the 12 layers (100 uL each) above the red blood cell layer following L-PRF protocol. Notice the massive increase in platelets (roughly a 20 fold increase specifically at the buffy coat layer between the yellow and red blood cell layers. Interestingly, all cells seem to accumulate within 3-5 layers (300-500uL) above the red blood corpuscle only.

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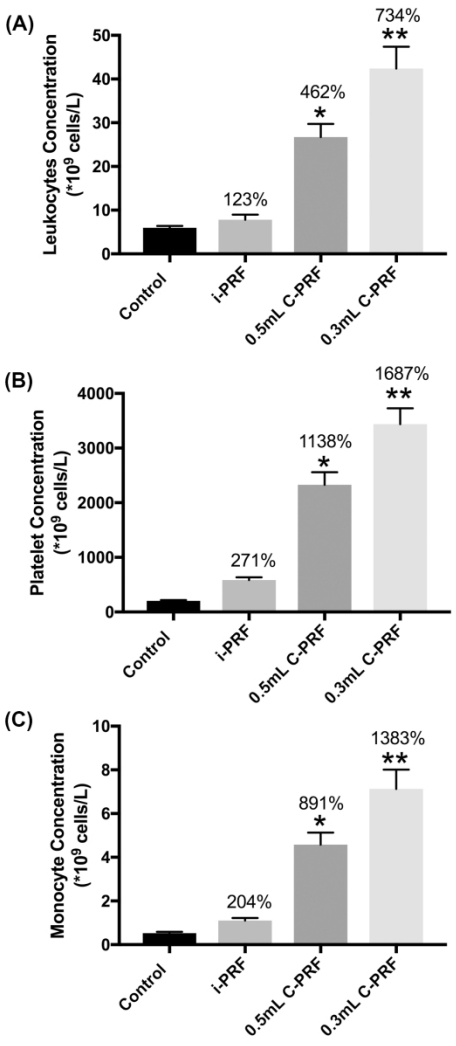

Figure 6

Figure 6: Concentration of (A) leukocytes (B) Platelets and (C) Monocytes following centrifugation using i-PRF protocols versus collecting 0.3-0.5 mL of concentrated-PRF (C-PRF). Notice that while i-PRF was typically responsible for a 1.2-2.5 fold increase in the various cell types following centrifugation, up to a 15 fold increase in platelet concentration could be achieved with C-PRF.

Table 1: Leukocyte, platelet and monocyte concentrations in whole blood, i-PRF, 0.5mL of C-PRF and 0.3mL of C-PRF (represented as 10^9 x cells/L). The final 3 columns represent the % increase in leukocyte, platelet and monocyte values when compared to baseline controls. Notice that while i-PRF protocols are able to achieve a roughly 1.2 to 2.7 fold increase in cell-types, C-PRF protocols, especially the 0.3mL C-PRF was able to massively concentrate leukocyte (>7 fold), platelets (>16 fold) and monocyte (>13 fold) when compared to controls.

	Whole Blood	i-PRF	0.5mL C-PRF	0.3mL C-PRF	i-PRF % increase	0.5mL C-PRF % increase	0.3mL C-PRF % increase
Leukocytes	6.0	7.8	26.7	42.4	123.8	461.7	733.7
Platelets	206.3	586.0	2327.9	3437.6	270.6	1138.2	1687.3
Monocytes	0.5	1.1	4.6	7.1	203.8	891.5	1383.4